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We have used brief bursts of relatively low intensity ultrasound (US) to alter the excitability of myelinated fibers within the frog sciatic nerve. The magnitude and direction of these changes are critically dependent on the timing of the burst relative to the electrical stimulus and are different for various fiber types and frog species. These effects cannot be emulated using equivalently timed electrical "pre-stimuli" and cannot be attributed to electrode artifacts. Since temperature rises of less than 0.01°C accompany effective US bursts and the levels are far below those causing cavitation, the effect is thought to be of a direct "micromechanical" nature. A selective activation or repression of slow conductance channels would, at this jucture, appear to be the most plausible explanation for these effects.

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PROGRESS REPORT ON CONTRACT N00014-87-K-0313

PRINCIPAL INVESTIGATOR: Howard Wachtel

CONTRACTOR: University of Colorado

CONTRACT TITLE Electrochemical and Micromechanical Models of Neural Membranes

START DATE: 1 April 1987

RESEARCH OBJECTIVE: To utilize pulsed and CW acoustic fields over a broad range of ultrasound and audio frequencies to selectively effect channel function in neuronal membranes, and from the pattern of such effects, to educe models of channel structure.

PROGRESS (Year I): Most of this first year was spent developing the exposure methodology and neurophysiological monitoring techniques necessary for exploring the effects of ultrasound pulses on isolated neural tissue. The ultrasound exposure system we developed was based on the use of wide band transducers which focused energy within an aperture as small as 2mm. The basic system, using a single transducer, can be upgraded to include three or four transducers, covering different frequency bands, arranged in a "turret" so that a very wide range of sonic and ultrasonic frequencies could be covered in a single experiment with only minor interruptions. The determination of field intensity produced within tissue placed over the aperture turned out to be a fairly complex problem and has been approached, but not concluded, in two ways. The first technique was essentially calorimetric. Using extremely wide (several seconds) pulses temperature rises on the order of several °C were generated and measured. Extrapolation of these measurements back to the narrower (msec.) pulses used in the actual exposure showed that temperature rises of less than 0.01°C accompanied the actual exposures (which only lasted 1 msec or less). The other technique for monitoring ultrasound intensity involves the use

of a wide band hydrophone. However, the calibration of this device in terms of pressure amplitudes, is not provided by the manufacturer and will have to be undertaken as part of this project.

Our initial choice for the isolated neural system to be studied was the gastropod ganglion, extracted from snails such as Aplysia or Lymnea (as is indicated in Figure 1). It proved to be technically quite difficult to position such ganglia directly over the aperture while keeping them stable enough for microelectrode penetration. We were also concerned with the possibility that the ultrasound field might affect the microelectrode in such a way as to produce mechanical artifactual effects on the neurons penetrated. For these reasons, we put aside the ganglion-microelectrode approach in favor of using a compound nerve trunk (the frog sciatic nerve) which could be stimulated and recorded from using external electrodes. The recording electrodes were placed well outside the ultrasound exposure aperture. The most pronounced effects are observed when the stimulating electrodes are placed within the field, but analogous results were also obtained when they were located beyond the aperture. Direct effects of the ultrasound on the electrodes were thus largely eliminated as a confounding factor in interpreting the neural responses, especially in view of the observation of the greatest effects occurring at temporal points well beyond the termination of the ultrasound pulse.

The most interesting result we have seen so far is the temporal selectivity of the ultrasound pulse (as shown in Figure 1). A marked depression of a half maximal compound action potential (CAP) is seen when the US pulse is delivered about 7 milliseconds prior to the electrical stimulation of Type A fibers. This inhibitory "window" extends from about 3 to 12 msec, but longer delays of about 25 msec produced a slightly enhanced CAP, and even longer delays, approximating 35 msec, constituted a secondary inhibitory "window." This temporal profile was quite consistent for all Type A fiber from the same frog species. However, a different profile was seen for the slower conducting Type B fiber (Figure 1). Here the shortest delays led to enhanced CAPs while the most marked depression was seen for a 14 msec. delay (from the US pulse to the electrical stimulation). Over a wide range of ultrasound pulse

energies the suppressive effect on the CAP was linear, as shown in Figure 2. However, a distinct threshold as well as a "saturation" level gives this "dose response" curve a sigmoidal character.

These effects could not be elicited using an electrical "prestimulus" instead of the ultrasound pulse, and the US pulse alone could not fire the nerve (even on rebound from long exposures). Furthermore, the very low temperature rises accompanying each pulse (less than 0.01°C) rules out the possibility that these effects are of a "first order" thermal nature. At these intensities we can also rule out cavitation as a contributing factor. It is possible that microthermal effects resulting from a high rate of temperature rise (several °C per second) could be involved. However, there is no evidence from previous studies to support this notion. At this point it seems to us that we are dealing with a fairly direct effect of the ultrasound on electrically excitable channels - albeit very slow ones. It thus appears that our original hypothesis that moderate levels of ultrasound would interact with ionic channels on a "micromechanical" basis is a viable one, and it could lead to an improved understanding of the structure of such channels.

WORK PLAN FOR YEAR 2: The most pressing item on our research agenda is to clearly define the ultrasonic fields that are induced in the nerve. To accomplish this we will carry out more precise calorimetry as well as field intensity measurements with a calibrated hydrophone. We will also estimate the distribution of the field within the nerve trunk using a multi-compartment model.

We also plan to monitor the activity of single fibers within the sciatic nerve which would give us a more intimate picture of these effects than does the CAP. Depending on how well this approach works, we may or may not attempt to carry out versions of this experiment on "giant axons" such as those obtainable from lobsters or other invertebrates. Ultimately (perhaps year 3) it may prove feasible to carry out such experiments under voltage clamping control which would give us a far more detailed picture of channel behavior.

Another approach we hope to initiate in year 2 would entail the use of channel blockers and other pharmacological agents. By comparing or modifying the effect of such agents with ultrasound the neurochemical basis for these phenomena may be explored.

Finally, our agenda for year 2 or year 3 would include a return to the isolated ganglion preparation in order to see if analogous effects can be demonstrated on pacemaker neurons, synaptic potentials, etc.

INVENTIONS: The neural phenomena we have seen suggests that ultrasonic pulsing devices could be used for anesthetic or analgesic purposes or perhaps to modify the effects of pharmaceutical or neuro-toxic agents. Such application would lend themselves to patentable inventions which we would like to explore with the ONR Patent Department.

PUBLICATIONS AND REPORTS: Two papers dealing with methodological and theoretical aspects of this project were presented in November 1987 at the EMBS meetings in Boston:

1. Electrical and Mechanical Forces on Membranes
   F. S. Barnes, H. Wachtel, R. Mihran

2. Effects of Wide Microwave Pulses on Isolated Nerve Cells
   H. Wachtel, F. Barnes

A manuscript dealing with the specific results on the frog sciatic nerve is now in preparation towards a September 1988 submission:

"Temporal Selectivity of Ultrasonic Pulses Capable of Modifying the Excitability of Myelinated Axons"
R. Mihran, H. Wachtel, F. Barnes

TRAINING ACTIVITIES: Two graduate students are currently supported by this ONR contract:

Richard Mihran is a Ph.D. candidate whose dissertation will, most likely, be based on these studies.

Donna Roberts is an M.S. candidate and is likely to base her thesis on some aspect of this study.

Women or minorities	1
Non Citizens	0

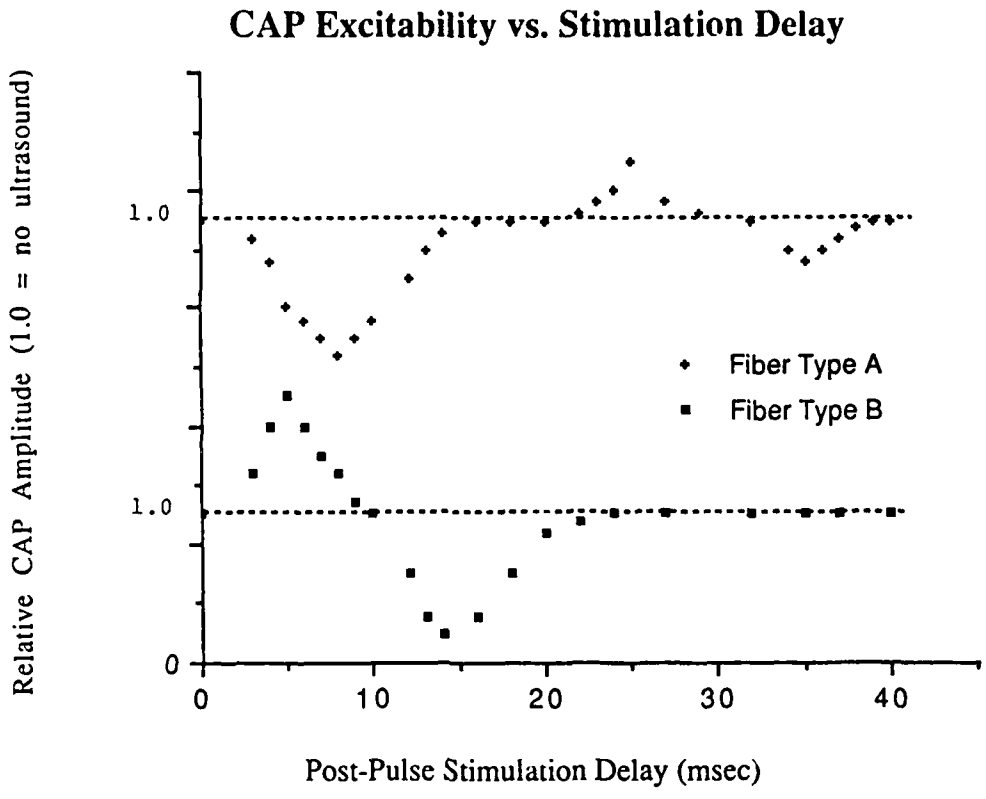

Figure 1: The effect of sciatic nerve excitability of a nominal 500 μsec ultrasound pulse (2 Mhz) of subthermal energy. A relative excitability of 1.0 indicates Compound Action Potential (CAP) amplitude in the absence of any applied ultrasound. Maximum effects are seen to occur at distinct temporal windows, i.e., delay points where an electrical stimulation of fixed amplitude and duration will elicit greater or lesser excitation than the no-ultrasound condition, as indicated by the relative amplitude of the resulting CAP. Distinct windows are associated with the major classes of fiber types present in the sciatic bundle. With fiber type A, shown as the upper plot of Figure 1, maximum suppression of the CAP occurs when electrical stimulus follows on ultrasound pulse with a 7 msec delay. A secondary suppression occurs at 35 msec, while maximum enhancement of excitability occurs at 24 msec. Fiber class B exhibits significant enhancement when electrical stimulus follows the ultrasound by 4.5 msec, and suppression at 14 msec as shown. Notably, little or no effects are observed when electrical stimulus occurs within the duration of the ultrasound pulse.

CAP Suppression as a Function of Ultrasound Pulse Energy

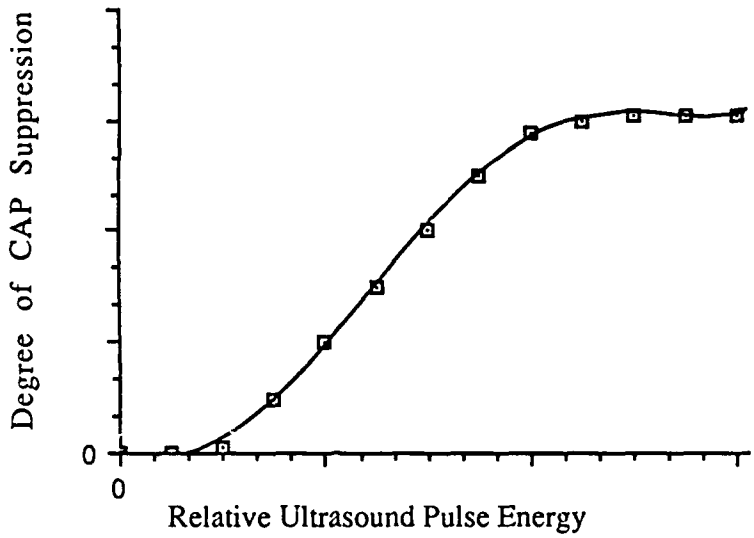

Figure 2: Plot of the Compound Action Potential suppression as a function of relative ultrasound pulse energy (Relative intensity × duration) for the 14 msec delay point of a group of B type fibers. Note that a minimum pulse energy is required before any suppression is observed. At greater pulse energies, the degree of suppression follows approximately a linear course, until a point of plateau where greater pulse energies do little towards increasing the effect. This form of pulse energy vs. effect is consistent generally with the other suppression or enhancement points of both fiber classes.